

# Growing Incidence of Non-Dermatophyte Onychomycosis in Tehran, Iran

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## Abstract

**Background:** Non-dermatophyte onychomycosis (NDO) is caused by a wide range of mold fungi other than dermatophytes, and has been reported at various rates in different countries worldwide. Studies on the incidence of NDO in the community are essential for understanding its epidemiology and control, as well as for the appropriate treatment of these infections.

**Objectives:** In this study, the incidence of NDO in Tehran, Iran, was compared to the incidence of onychomycoses due to dermatophytes and yeasts.

**Methods:** From 2014 through 2015, samples from a total of 1,069 patients with suspected fungal nail diseases, who were referred to three medical mycology laboratories in Tehran, were collected and subjected to direct examination (all samples) and culture (788 samples). Differentiation of the causative agents of onychomycosis was based on microscopic observation of characteristic fungal elements in the nail samples and growth of a significant number of identical colonies on the culture plate.

**Results:** Based on only direct microscopy, onychomycosis was diagnosed in 424 (39.6%) cases, among which 35.8% were caused by dermatophytes, 32.7% by yeasts, and 29.3% by non-dermatophyte molds (NDMs), while 2.2% were mixed infections. Direct exam was significantly more sensitive than culture for the diagnosis. The most commonly isolated NDMs were *Aspergillus* spp. (69.3%, n = 52), followed by *Fusarium* spp. (n = 7). The other isolated species were *Paecilomyces* spp., *Scopulariopsis* spp., *Acremonium* spp., *Cladosporium* spp., and *Chrysosporium* spp., with only one case of each.

**Conclusions:** An increasing frequency of NDO compared to onychomycosis due to other causative agents has been noticeable over the past few years in Iran. This epidemiological data may be useful in the development of preventive and educational strategies.

**Keywords:** Onychomycosis, Non-Dermatophyte Molds, Epidemiology

## 1. Background

According to the international society for human and animal mycology (ISHAM), onychomycosis is an invasive fungal infection of the nails, without regard to the causative agent (1). This disease may involve the toenails or fingernails, and represents approximately 30% of superficial mycoses (2). It is the most common disease of the nails, constituting approximately half of all nail abnormalities, with an increasing incidence with age (3). The causative agents of onychomycosis include dermatophytes, yeasts, and non-dermatophyte molds (NDMs) (4). The term tinea unguium is reserved for onychomycosis caused by dermatophytes; however, tinea unguium and onychomycosis are sometimes considered synonymous. In this article, the term onychomycosis refers to infections caused by either dermatophytes, yeasts, or NDMs.

The estimated prevalence of onychomycosis is more

than 10% in the general population and 40% in elderly individuals, probably due to suboptimal immune function, inactivity, and the inability to maintain good foot care (5). It has been reported that the majority of onychomycoses are caused by dermatophytes, while yeasts and NDMs each account for approximately 10% of onychomycosis cases worldwide (3). Non-dermatophyte onychomycosis (NDO) is caused by hyaline (6, 7) and dematiaceous (8, 9) filamentous fungi that are commonly found as soil saprophytes or plant pathogens. Unlike dermatophytes, they are generally not keratinolytic (10). They live on the unkeratinized intercellular cement of the host tissue and must take advantage of previous keratin destruction by dermatophytes, trauma, or another nail disease. For this reason, they are sometimes considered secondary invaders of the nail plate (11). In Iran, a significant percentage of onychomycosis is caused by non-dermatophytes (12-14).

Although the list of NDM species that have occasionally been isolated from nails is quite long, only a few are regularly identified as real causes of onychomycosis. These include *Scopulariopsis brevicaulis* spp., *Fusarium* spp., *Acremonium* spp., *Aspergillus* spp., and *Scytalidium* spp. (15).

The epidemiological profiles of the causative agents of onychomycosis tend to alter over time, due to climatic, environmental, or socioeconomic factors, and can also be influenced by tourism (16). This variation may also reflect geographic differences in mold distribution, differences in the criteria used for diagnosis, and/or the use of mycological media for mold growth. Differentiation of the isolates is clinically important for targeted therapeutic decisions and for the prognosis, and for epidemiological purposes.

## 2. Objectives

The aim of this study was to determine the overall prevalence of dermatophytes, yeasts, and NDMs as the causative agents of suspected onychomycosis in patients in Tehran, Iran. This study is one of the large-scale reports done in a short period (one year) on the microbial epidemiology of onychomycosis in Iran.

## 3. Methods

### 3.1. Study Population

A total of 1,069 nail-clipping specimens were obtained from outpatients with suspected onychomycosis in Tehran during 2014 - 2015. The patients had been referred to three medical mycology laboratories for routine diagnostic procedures.

### 3.2. Mycological Examination

After cleaning the affected area with 70% ethanol, nail scrapings were collected from the deepest part of the nail and as close as possible to the intact parts of the nail by scraping the nail bed, the underside of the nail plate, and the hyponychium. One piece of each collected nail fragment was examined using a potassium hydroxide (KOH 20%) preparation to identify the presence of any fungal elements, including hyphae, arthrospores, yeast cells, and pseudohyphae. For a total of 788 samples, another part of the nail was cultured in plates containing Sabouraud dextrose agar (Difco, Detroit, MI, USA), with and without 0.05% cyclohexamide and 0.005% chloramphenicol, by inoculation of sample fragments onto the three points of an agar plate and incubating them at 28°C for 1 - 4 weeks. The cultures were checked twice weekly for evidence of growth. No growth at the fourth week was considered a negative culture. The criteria for a diagnosis of NDM onychomycosis

was made based on nail abnormalities consistent with this diagnosis, a positive KOH preparation with the presence of specific hyphae in the nail keratin, and, when the culture was done, the failure to isolate a dermatophyte in the culture and growth of identical mold colonies in the inoculation sites of the culture media. Samples with characteristic saprophytic hyphal elements on direct microscopy and significant growth of NDMs on culture were considered for species identification by colony morphology and a microscopic examination with lactophenol cotton blue preparation according to identification keys (17).

### 3.3. Statistical Analysis

Demographic and microbiologic data were analyzed using the SPSS (version 21.0) statistical package (18).

## 4. Results

The study population comprised 643 females and 426 males. All nail specimens were subjected to direct microscopic examination, while cultures were also performed on 788 (73.7%) of the samples based on physicians' requests. According to the microscopic tests, the prevalence of onychomycosis was 39.6% (n = 424), found in 185 males and 239 females. Fingernail onychomycosis was recognized in 38.3% of the cases, toenail onychomycosis in 59.1%, and a combination of both in 2.6%. Fingernail onychomycosis was significantly more prevalent in females than in males (120 versus 42), while toenail infections were significantly more common in males than in females (136 versus 115).

A total of 152 (35.8%) of the 1,069 samples showed the branching mycelium and/or arthroconidia representative of dermatophytes, 139 (32.7%) showed the blastoconidia and pseudohyphae representative of yeasts, and 124 (29.3%) showed the saprophytic mycelia representative of NDMs. Mixed infections were observed in 2.2% (n = 9) of the positive cases (Table 1). Various clinical forms of onychomycosis due to NDMs were seen among the samples; examples are shown in Figure 1. The causative factors for fingernail onychomycosis were *Candida* spp. (66.2%), dermatophytes (10.4%), NDMs (21%), and mixed infections (2.4%). The rates of the same factors for toenail onychomycosis were 10%, 52.8%, 35.2%, and 2%, respectively.

The results indicated that laboratory confirmation was achieved through direct examination with cultures in 726 samples, and by only a positive direct exam in 45 cases or only a positive culture in 17 cases (Table 2). The most commonly isolated NDO was *Aspergillus* spp. (69.3%, n = 52), followed by *Fusarium* spp. (n = 7). There were fewer isolated *Paecilomyces* spp., *Scopulariopsis* spp., *Acremonium* spp., *Cladosporium* spp., and *Chrysosporium* spp., each with only one

case (1.3%). The causative agents in 11 of 75 cases of NDM onychomycosis were not diagnosed with culture colonies.

## 5. Discussion

Correctly determining the etiologic agents of onychomycosis is important in order to provide a baseline for administering appropriate antifungal therapy and identifying the source of infection, hence facilitating prevention measures. An inaccurate clinical diagnosis may prolong the patient's discomfort and result in a financial burden due to expensive antifungal therapy (19).

In the present study, the incidence of onychomycosis was confirmed in 39.6% of the examined patients. Although a higher prevalence of onychomycosis was reported in other studies conducted in different regions of Iran, such as Sari (56.8%) (14), Khoozestan (42.9%) (20), and Kermanshah (45.2%) (13), the incidence in our study was more than in some older Iranian studies, such as those by Asadi et al. (18.9%) (21) and Moghaddami et al. (28.9%) (22). In our samples, onychomycosis affected toenails (59.1%) more often than fingernails (38.3%), probably due to toenails' slow growth, which facilitates the invasion of the fungus and is perhaps supported by factors such as trauma and poor circulation (23). Also, onychomycosis affected more females (56.4%) than males (43.6%) in the present study. A higher incidence of onychomycosis in women has been reported in other studies (24-26).

The etiological fungal agents were dermatophytes (35.8%), yeasts (32.7%), NDMs (29.3%), and mixed infections (2.2%) in the present study. Other local studies conducted in the cities of Ghazvin (26) and Tehran (27) showed that dermatophytes were the major causative pathogens. Similarly, in studies performed in Mexico and Malaysia, dermatophytes were the principal pathogens (28, 29). Nevertheless, the epidemiology and etiology of onychomycosis varies in different geographic areas, as summarized in Table 3.

Unlike many studies performed in Iran (13, 14, 20), in the present survey, the frequency of onychomycosis caused by NDMs was almost equal to the frequency of nail infections caused by dermatophytes and yeasts. Among the studies done in Tehran, the frequency difference between the most common causes of onychomycosis (dermatophytes or yeasts) and NDMs was 35% - 64% (12, 22, 27, 30). This difference was only 6% in our study, which is similar to a previous study carried out in Tehran in 2009 (25).

The prevalence of NDMs isolated from nail infections in various parts of the world ranges between 1.49% and 33.5% (30-33); however, it seems that this rate has increased dramatically in the past several years (34, 35). Although our study is not a comprehensive epidemiological survey

and we did not test all samples, these random data demonstrate an increasing occurrence of onychomycosis due to NDMs. This study demonstrated that 29.3% of unusual onychomycosis cases are due to NDMs, which is 1.5 times more than the 19% found in the last study conducted in Tehran (2010) (12).

The increased incidence of NDOs may be due to the widespread use of broad-spectrum antibiotics and the increased frequency of immunosuppression, chemotherapy, debilitating diseases, metabolic diseases such as diabetes, occupational accidents, aging of the population, and any other factors that predispose the nails to the invasion of pathogens. Thus, NDMs should be considered important pathogens, with a high index of suspicion in evaluating patients with cultures that are negative for dermatophytes, or in those experiencing treatment failure (10). Non-dermatophyte onychomycosis presents clinicians with a greater diagnostic challenge compared to dermatophyte onychomycosis. The latter can be diagnosed with the single isolation of a dermatophyte, but NDM onychomycosis requires further measures for confirmation (36).

The prevalence of the fungi responsible for NDOs varies considerably in different studies reported in the literature. In general, the top five organisms in terms of published confirmed isolates worldwide are *Scopulariopsis brevicaulis*, *Fusarium* spp., *Aspergillus* spp., *Scytalidium dimidiatum*, and *Acremonium* spp. According to the data in the present survey, the overall prevalence of NDM onychomycosis due to *Aspergillus* spp. is 69.3%. Although a study conducted in Tehran in 2001 (27) reported that *Scopulariopsis brevicaulis* spp. were the most common agent of NDO, other studies carried out in different areas of the country (13, 14, 37), including Tehran (12, 22), revealed that a large percentage of NDMs are *Aspergillus* spp., particularly *A. flavus*. In recent years, onychomycosis caused by different *Aspergillus* species has increased, as evidenced by case reports and epidemiological studies (38, 39). It is noteworthy that there was a case of a nail infection by *Chrysosporium* among our samples, which is the first confirmed case of onychomycosis caused by this species in Iran.

The prevalence found in this survey of onychomycosis due to NDMs was higher in toenails (77.7%) than in fingernails (22.3%), which is similar to results reported by Nouripour et al. (70.3%) (40), Khosravi et al. (87.5%) (27), and Zaini et al. (80%) (25). In contrast to our observation, an epidemiological survey in Khoozestan, southwest Iran, noted that NDMs were higher in fingernails than in toenails (20).

In conclusion, NDO appears to be an increasing problem in Iran, with a growing trend of NDMs isolated from onychomycosis compared to other causative agents of onychomycosis, noticeable in our samples and in other recent studies in Tehran. Since the published data on NDMs are

**Table 1.** Frequency Distribution and Causative Agent of Onychomycosis Diagnosed by Mycological Examination and/or Culture

	Dermatophyte (Finger/Toe)	Candida (Finger/Toe)	Mold (Finger/Toe)	Mix (Finger/Toe)	Negative (Finger/Toe)	Total (Finger/Toe)
By direct examination	152	139	124	9	645	1,069
By culture	94	101	75	12	506	788

**Table 2.** Comparison of Direct Microscopy Examination and Culture Results for 788 Nail Samples<sup>a</sup>

	Culture-Positive	Culture-Negative	Total
Microscopy-positive	265 (33.6)	45 (5.7)	310 (39.3)
Microscopy-negative	17 (2.2)	461 (58.5)	478 (60.7)
Total	282 (35.8)	506 (64.2)	788 (100)

<sup>a</sup>Values are expressed as No. (%).

**Table 3.** Review of Studies Performed on Onychomycosis in Iran With Regard to Causative Agents

Year	City	Total Samples (n)	Prevalence of Onychomycosis (%)	Prevalence of NDMs (%)	Prevalence of Dermatophytes (%)	Prevalence of Candida spp. (%)	Commonest NDM spp.	Other isolated NDMs	Reference
1989	Tehran	927	28.9	1.86	32.1	66.04	<i>Aspergillus</i> spp.	<i>Penicillium</i> spp.	(22)
2000	Khoozestan	2,525	42.9	2.1	10.4	87.5	<i>A. fumigates</i>	<i>A. niger</i> , <i>Scopulariopsis brevicaulis</i> , <i>Fusarium</i> spp., <i>A. flavus</i> , <i>Alternaria</i> spp., <i>A. terreus</i> , <i>Mucor</i> spp.	(20)
2001	Tehran	115	84.3	8.2	48.4	43.3	<i>S. brevicaulis</i>	<i>Aspergillus</i> spp., <i>Acremonium</i> spp., <i>F. solani</i>	(27)
2002	Tehran	252	39.9	14.2	31.8	54	<i>A. flavus</i>	<i>A. niger</i> , <i>Scopulariopsis brevicaulis</i> , <i>Fusarium</i> spp.	(30)
2009	Kashan	137	18.9	23	34.7	42.3	<i>A. flavus</i>	<i>A. fumigatus</i> , <i>Scopulariopsis</i> spp., <i>Fusarium</i> spp.	(21)
2009	Tehran	549	47.9	32.6	21.9	45.5	<i>A. flavus</i>	<i>A. fumigates</i> , <i>A. niger</i> , <i>Penicillium</i> spp., <i>Rhizopus</i> spp., <i>Cladosporium</i> spp., <i>Acremonium</i> spp.	(25)
2010	Esfahan	488	39.8	28.4	13.9	57.7	<i>A. flavus</i>	<i>A. nidulans</i> , <i>A. fumigates</i> , <i>Acremonium</i> spp., <i>Cladosporium</i> spp., <i>Scopulariopsis brevicaulis</i> , <i>Fusarium</i> spp., <i>Penicillium</i> spp.	(24)
2010	Tehran	504	42.8	19	21.3	59.7	<i>A. flavus</i>	<i>Aspergillus</i> spp., <i>Fusarium</i> spp., <i>Penicillium</i> spp., <i>Scopulariopsis</i> spp.	(12)
2010	Qazvin	308	40.2	3.2	50.2	46.6	<i>A. niger</i>	<i>A. flavus</i>	(26)
2013	Kermanshah	1,086	45.2	2.9	18.6	78.5	<i>A. flavus</i>	<i>Aspergillus</i> spp.	(13)
2014	Sari	1,100	56.8	15	23	62	<i>A. flavus</i>	<i>A. fumigatus</i> , <i>Fusarium</i> spp., <i>Scopulariopsis brevicaulis</i> , <i>Geotrichum</i> spp., <i>Trichosporon</i> spp., <i>Cladosporium</i> spp., <i>Penicillium</i> spp.	(14)

limited, more studies on this group of fungi are recommended to clarify aspects of its epidemiology and pathogenesis.

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**Figure 1.** Examples of Nails Affected by Onychomycosis Caused by Different Species of NDMs



(A) to (F) are infections due to *Aspergillus terreus*, *A. niger*, *Fusarium* spp., *Chrysosporium* spp., *Acremonium* spp., and *Scopulariopsis* spp., respectively.

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